Docket No.: 066662-0092 **PATENT**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	:	Customer Number: 41552
Palsson, Bernhard	:	Confirmation Number: 1729
Application No.: 09/923,870	:	Group Art Unit: 1631
Filed: August 06, 2001	:	Examiner: Negin, Russell Scott

For: METHODS FOR IDENTIFYING DRUG TARGETS BASED ON GENOMIC SEQUENCE DATA

DECLARATION UNDER 37 C.F.R. § 1.132

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Jay D. Keasling, declare as follows:

1. I am a Professor in the Department of Chemical Engineering and Bioengineering at the University of California, Berkeley (UC Berkeley). I also hold the Hubbard Howe Jr. Distinguished Professor of Biochemical Engineering. I am the Acting Deputy Director of the Lawrence Berkeley National Laboratory and Synthetic Biology Engineering Research Center and am CEO of the Joint BioEnergy Institute. I joined the faculty of UC Berkeley in 1992 as an Assistant Professor. I became an Associate Professor in 1998 and was elevated to full professor in 2001. I served as Vice Chair of the Department of Chemical Engineering from 1999-2000 and have served as the Director and an Executive Committee Member of the UC BioSTAR Program since 2000.

2. Prior to joining the UC Berkeley faculty I obtained a Bachelors of Science majoring in chemistry and biology in 1986 from the University of Nebraska-Lincoln. I earned my Masters degree in 1988 and my Ph.D. in 1991, both in chemical engineering from the University of Michigan. From 1991-1992 I did a postdoctoral fellowship at Stanford University

EXHIBIT A

in Biochemistry. A copy of my curriculum vitae and a list of publications is attached as Exhibit 1.

3. I am an inventor or co-inventor on at least four U.S. patents and 16 U.S. applications. I am a founder of Amyris Biotechnologies and serve as the Chair of its Scientific Advisory Board. Amyris focuses on the microbial production of renewable fuels. I also am a founder of LS9 and Codon Devices. I have been a member of Genomatica's Scientific Advisory Board for the past year. My accomplishments in the fields of chemical engineering and synthetic biology have been reported in Time and Newsweek, and Discover magazine named me as the Scientist of the Year in 2006 for my work in synthetic biology, including treatments for malaria, AIDS, and cancer as well as discoveries of new fuel resources.

4. I am very familiar with stoichiometric models of metabolism and have read U.S. application serial no. 09/923,870, by Palsson. I also am very familiar with Dr. Palsson's work, including the publication that is the basis of this application (Edwards and Palsson, *Proc. Natl. Acad. Sci. U.S.A.*, 97:5528-33 (2000)). I understand that the invention described in this application is directed to constructing genome specific stoichiometric matrices that can be utilized with flux balance analysis for modeling metabolism. The application claims, in part, a method of simulating a metabolic capability by incorporating metabolic reactions through the use of genome information to assign function to metabolic proteins of unknown function.

5. I have read the Office Action mailed December 18, 2008. I understand that the claimed invention stands rejected for obviousness over the combination of references to Pramanik and Keasling., *Biotech. and Bioengineering* 56:398-421 (1997) in view of Blattner et al., *Science* 277:1453-69 (1997) and in view of Kunst et al., *Rev. in Microbiol.* 142:905-12 (1991). The Examiner appears to rely on Pramanik and Keasling for describing a stoichiometric model of *E. coli* metabolism and then combines it with Blattner et al. and Kunst et al., reporting the sequencing of *E. coli* and *B. subtilis* genomes, respectively, to conclude obviousness. The sequencing papers are used to support the Examiner's argument that one would have expected to be able to determine the function of genes encoding proteins of unknown function based on sequence comparisons with a different organism.

6. Pramanik and Keasling, the primary reference cited in the above rejection, is a publication from my laboratory and I am very familiar with this work. At the time of Dr. Palsson's invention, the *E. coli* genomic sequence had become available and my laboratory was actively working with stoichiometric models, including the model described in Pramanik and Keasling. We did not consider incorporating additional reaction information into the model based on the genomic sequence results for at least two reasons.

7. First, *in silico* models of metabolism such as that described in Pramanik and Keasling are complex computational models that are only as accurate as the information one includes in the model. There are a large number of metabolic enzymes encoded in the genome that are not used in metabolism. Incorporation of reactions based on genomic information would have included such unused enzymes and reactions in the model. One would have expected a large number of inaccurate fluxes to occur that would, in effect, travel everywhere throughout the network (i.e., wild or uncontrolled fluxes). As a result, the model would not have been predictive of an organism's metabolism and would have been expected to be much less accurate than the model described in Pramanik and Keasling. The fact that Dr. Palsson was able to construct a model incorporating reactions based primarily on genomic sequence information was surprising and unexpected because it did not result in wild fluxes nor decrease in accuracy compared to the Pramanik and Keasling model. Rather, the model yielded results that reflected actual cellular metabolism and was predictive despite the inclusion of more enzymes than what the network uses.

8. Second, incorporating reactions based on homology comparisons of unknown genes with metabolic genes in other organisms also was expected to yield inaccurate results. Although sequence identity comparisons can be predictive there are examples where identifications have been incorrect. Therefore, identification and assignment of some open reading frames as a putative metabolic enzyme was speculative and likely resulted in some incorrect assignments. These incorrect assignments can result in the inclusion of multiple reactions carrying out the same reaction, inclusion of unused reactions and the inclusion of non-metabolic enzymes into the metabolic network. For the reasons described above, incorporation of such inaccurate information was expected to generate wild fluxes if incorporated into a model such as that described in Pramanik and Keasling. It was surprising that one could. in fact,

I have read the Office Action mailed December 18, 2008.

incorporate putative metabolic enzymes and produce results that are predictive of cellular metabolism. Hence, the actual result of the claimed method is unexpected because this method is able to accurately predict metabolism even though reactions are incorporated based on deductions from sequence comparisons.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that any such willful false statement may jeopardize the validity of the application or any patent issued thereon.

Jay D Keasling

12 June 2009

Jay D. Keasling

Date

SDO 140829-1.066662.0092